Experimental Studies on Cerebrovascular Spasm in Cats

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by

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INTRODUCTION

The concept of spasm of cerebral arteries is of particular importance as regards its relationship to the ruptured intracranial aneurysm. Since Reid in 1950[45] first reported an angiographic evidence of narrowing in the arteries near the ruptured aneurysm, such an arterial narrowing has been noted repeatedly in the cases of subarachnoid hemorrhage [6][16][19][25][31][35]. Such an arterial narrowing on angiograms was termed cerebral vasospasm and its clinical importance has been emphasized on the ground that its presence has a grave influence on the prognosis of patients irrespective of being treated by surgical or non-surgical means[2][3][25][31][40][43][46].

Intensive clinical and experimental studies have been performed on vasospasm, but its cause and mechanism have not yet well been understood. Many factors have been blamed as the cause of cerebral vasospasm. These include mechanical stimulations at the moment of aneurysmal bleeding[10][16][19][23][38][43], serotonin contained in the platelets[44], some unknown vasoconstrictors in the fresh blood[15], or vasoactive amines released during

fibrolysis of the blood clot, etc[5)51)]. On the other hand, some authors have cast doubt even upon the presence of vasospasm itself, speculating that an arterial narrowing might be due to some artefacts of an angiographic procedure[48)], or to the organic lesions in or around the arteries[47)].

In the present experiments, therefore, a systematc analysis has been made to elucidate which proposed factors are resposible for the production of cerebral vasospasm.

PART 1. MECHANICAL FACTORS IN THE PRODUCTION OF VASOSPASM

Method ; Ten adult cats unselected as to sex or size were anesthetized with intraperitoneal Nembutal (30 mg/kg). The head was immobilized in a stereotaxic instrument. A midcervical skin incision was made from the ramus of the mandibule to the sternum. After cannulation of the trachea, a selfretractor was placed between the esophagus, trachea, and ramus of the mandibular bone with the care not to press the carotid arteries, thus exposing the capitus muscle overlying the anterior atlanto-occipital junction and the lower portion of the clivus. Having been freed from the attached muscle, the clivus was removed with a rongeur from the rim of the foramen magnum to the level of the midpons. With the aid of Zeiss dissecting microscope, only the outer layer of the dura rich in vascular supply was stripped away with the use of a small hooked mess (cystitome) and meticulous attention was given to hemostais at this stage. With this precaution, there was no bleeding at the time when both the arachnoid and the inner layer of the dura were opened. The inner layer of the dura was reflected away laterally, but the arachnoid was not reflected widely in order to avoid mechanical stimulations to the vessels, for numerous avascular arachnoid bunds are present between the arteries and the arachnoid membrane. Insteads, multiple tiny incisions were made on the arachnoid membrane to ensure the access of the tested substances to the arterial wall. After exposure of the vertebral and basilar arteries, the field was filled with warm physiologic saline solution and left for at least half an hour to keep the arteries in the stable condition. Various mechanical stimulations were then applied to the basilar artery.

In some cats, the stimulations were also applied to the pial arteres over the cerebral convexities which were exposed through a parieto-temporal craniectomy. In these cases the arachnoid membrane was not stripped to avoid a tear of small branch arteries.

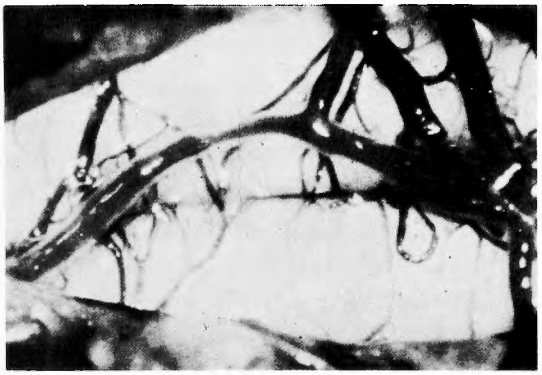
Photographs were taken of the vessels before and after the stimulation using Fuji color film by a 35 mm Zeiss camera attached to the operative microscope. Color picture were later projected onto the screen so that the vessels were carefully measured.

Results ; When the basilar artery was stroked with the tip of a glass rod, a severe beads-like constriction of the artery followed. Pinching of the vessel by a forceps resulted in a severe local vasoconstriction with a slightly dilated portion in the middle of its vasoconstricted region. Stretching of the vessel was made by stroking it at the right angle to its long axis. Such a stretching produced a marked diffuse vasoconstriction.

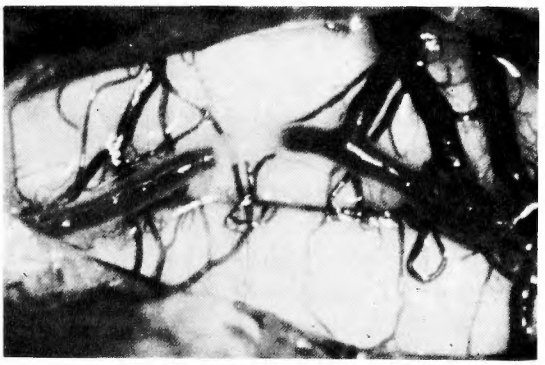
All these constrictions produced by mechanical stimulations usually occurred only over the area of the vessel stimulated. The duration of these vasospasm was usually about 3 to 15 minutes. In no cases did the vasospasm persist for more than thirty minutes.

In some cases, small opaque white bodies were observed in the artery at the point where severe mechanical stimuli were applied (Fig. 1). These bodies were seen at first adhering the wall of the artery, increased in size gradually, and then, suddenly detached and shot off into the blood stream. White bodies were again produced at the same portion of the artery in a few cases, but the vessel alway became clear without any remnant mass of the white bodies soon after the artery returned to its original size.

A similar vasospasm and sometimes a white body formation were induced by severe mechanical stimuli also in the exposed pial arteries in the parietotemporal region (Fig. 2).

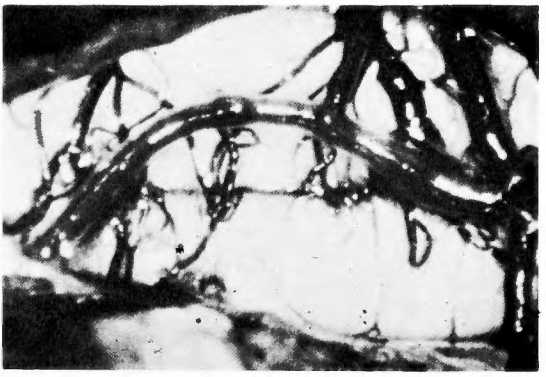


(a) Severe vasospasm was induced by mechanical stimulation in the basilar artery.

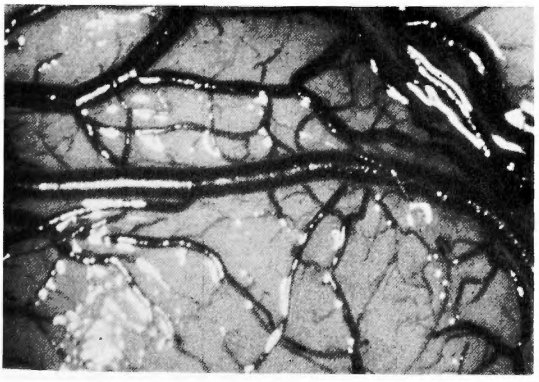


(b) White body obstructed the artery where a severe mechanical stimulation was applied.

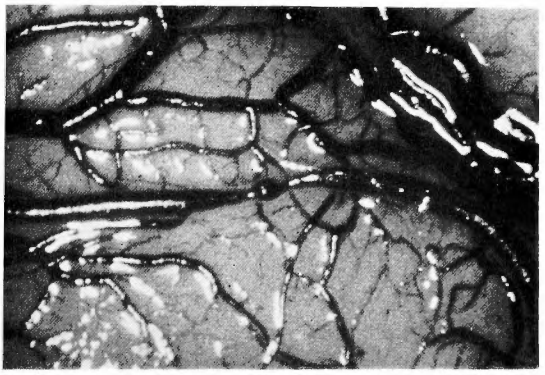
Fig. 1.



(c) 15 minutes after the stimulation, the white mass was dislodged and disappeared. Vasospasm was relaxed.



(a) The vessels in the parietal region (control).



(b) Severe vasospasm with a slightly dilated portion in the vasospastic artery. The dilatation probably resulted from a damage of the smooth muscle by a strong insult. Vasospasm relaxed ten minutes later.

Fig. 2.

Comment ; The arteries in the central nervous system are characterized by much thinner smooth muscle layers, thick elastic fibers and a prominent internal elastic membrane[6)12)]. These histologic features imply a relatively feeble contraction with a high degree of resiliency of the intracranial arteries.

However, a bulk of experimental studies[10)14)20)23)] including the present observation have shown that main cerebral arteries in the base of the brain as well as the small arteries over the cerebral convexities are able to constrict in response to mechanical stimulations. Furthermore, neurosurgeons have noticed a local spasm in the human arteries during operative manipulation[6)38)40)41)43)]. These facts indicate that vasospasm may be induced by mechanical stimulations at the time of aneurysmal rupture, in which the blood separates the artery from the brain surface and the arachnoid membrane, resulting in a traction of the artery via arachnoid bands bridging between them[34)].

However, the present experiment indicates that such a stretching of the artery does not seem to be a sufficient insult to cause the persistent "clinical" vasospasm, for vasospasm in response to mechanical stimuli was transient. Therefore, the persistent "clinical vasospasm" can not be attributed to a mechanical stimulation alone, although it may play some role in the very early stage of subarachnoid hemorrhage.

The intravascular white body formation has been frequently noted in the severely injured vessel[20)24)36)54)]. The white body was localized exclusively at the point where the vessel wall was severely injured, usually resulting in total obstruction of the vessel. Since vasospasm is an arterial narrowing, but does not cause total obstruction, of the vessel and since it persists for a longtime, it is most unlikely that such a white body can produce the arterial narrowing seen in the arteriograms of the patients with subarchnoid hemorrhage.

PART 2. VASOSPASM INDUCED BY FRESH SUBARACHNOID BLEEDING

Method ; The basilar artery was exposed transorally and was photographed as previously described. Subarachnoid hemorrhage was induced by a topical application of the fresh arterial blood or an incision of a small branch artery with care not to give mechanical stimulations to the parent artery. Although the field was obscured by the dripped arterial blood or the extravasated blood, a column of the blood within the vessel was outlined by the wall of the vessel as a white streak between the two red streaks. When the field was covered by too much of the blood, it was gently drawn by a siphonage function of cotton swabs or by a constant stream of warm saline solution. The results were expressed in per cent of the change in the vessel diameter relative to the control.

Histological examinations of the control and vasospastic arteries were performed as follows. Solution of liquid isopentane cooled at about -100°C was poured on the exposed basilar artery. At the same time, the cats were sacrificed by injection of Nembutal direcctly into the heart. The frozen basilar artery was punched out by a special round scouper together with some brain tissues underneath it and was dropped into the cooled isopentane for additional freezing. After they were fixed by freeze-drying in vacuo for 7 days at −40°C, they were embedded in paraffin blocks and serially sectioned at the

thickness of 7μ. They were stained with Hematoxylin-Eosin and Elastica Van-Gieson.

In three cast, a small cathetor was introduced from the femoral artery to the arch of the aorta, and arteriography was performed with injection of 5 cc of 76 % Urografin through the cathetor before and after incision of a small branch artery under direct observation of the exposed basilar artery through the operative microscope.

The response of the pial arteries on the cerebral convexity to a rupture of an artery was also studied in some cats. A topical application of the fresh blood to the pial arteries was not tried, for the arachnoid membrane was left and a direct contact of the blood with the arterial wall could not be expected.

Results ; A topical application of the fresh blood as well as subarachnoid bleeding by incision of a branch artery caused consistently an immediate diffuse constriction of the exposed arteries (Fig. 3). The basilar artery constricted to 33 to 70 per cent of its previous diameter (Table 1), and a white streak of the arterial wall increased in its width. In some cats, such a marked vasoconstriction of the exposed basilar artery resulted in a transient cessation of respiration for a a few minutes and artificial respiration was required. However, the vasospasm was seen only in the region where the arterial wall was irrigated with blood.

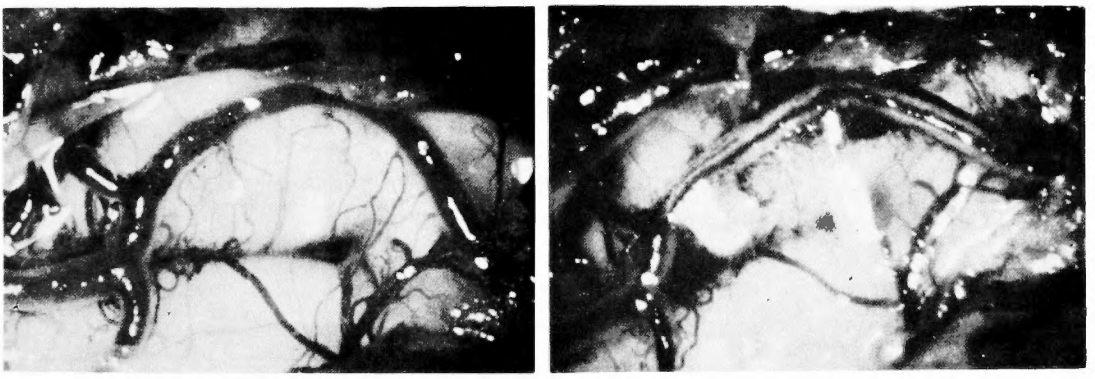


Fig. 3.

(a) The basilar artery (control).

(b) Severe vasospasm produced by rupture of a branch artery. The greatly widened white streaks of the arterial wall are noted between the extravasated blood and the column of blood in the artery.

Table 1 The caliber change of the basilar artery by means of topical application of the fresh blood, and subarachnoid bleeding by incision of a small branch artery

	Average	−60% −50% −40% −30% −20% −10% 0
Topical application of fresh arterial blood	−35%	
Subarachnoid bleeding induced by incision of a small branch artery	−46%	

When the extravasated blood from a cut branch artery was washed off by a rapid constant stream of warm saline solution, the artery remained normal in the caliber during active bleeding as shown in the Figure 4. When the irrigation of physiological saline solution was stopped, the arterial wall was stained with the extravasated blood and then, a diffuse vasospasm was produced. A severe vasospasm was always seen when the extravasated blood contacted directly with the arterial wall.

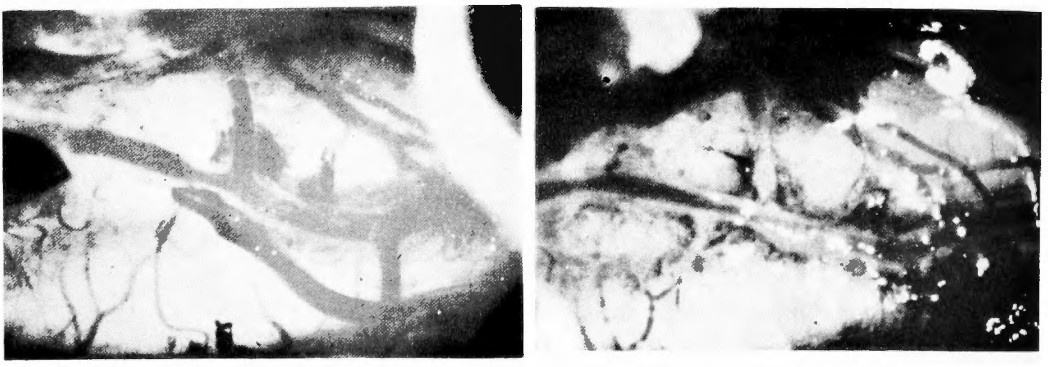


Fig. 4.

(a) The basilar artery is bleeding from a cut small branch artery. No vasospasm is noted.

(b) The basilar artery was bathed with the extravasated blood. Severe vasospasm occurred and the animal died.

When cooled isopentane solution was poured on the exposed basilar artery, the artery became white in color almost instantaneously, the wall of the artery being frozen immediately without a significant change from the state observed through an operative microscope.

The wall of the vasospastic basilar artery was characterized by the increased mural thickness in relation to the arterial diameter, the columnar endothelial cells, and the markedly convoluted internal elastic lamina with an irregular indentation and longitudinal folds. The smooth muscle layers greatly increased in width, and the smooth muscle cells were compressed and shortened with the nuclear deformation, especially in the layer

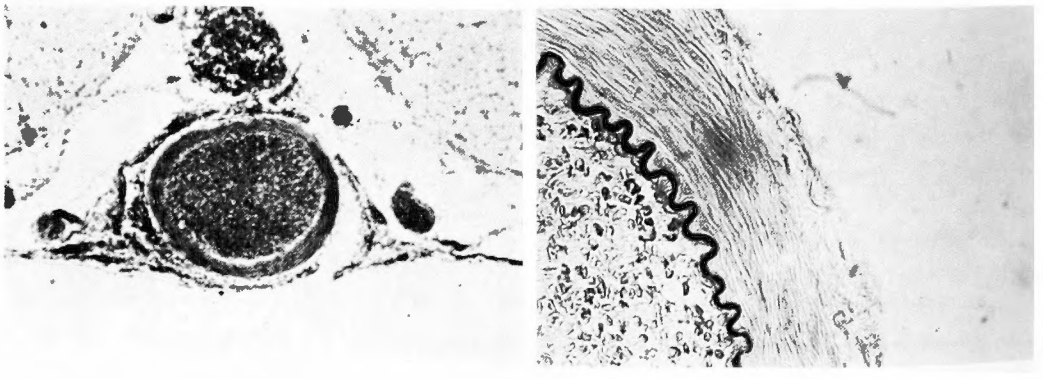
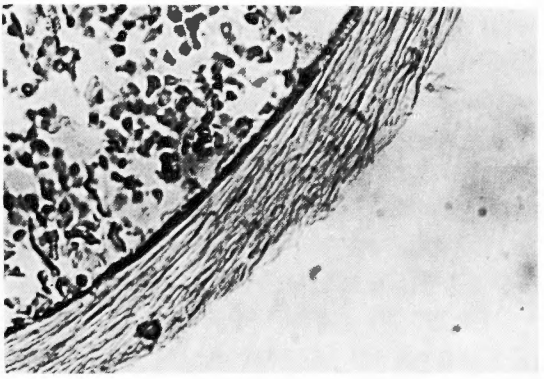


Fig. 5.

(a) Hematoxylin-Eosin stain of the vasospastic basilar artery.

(b) Elastic Van-gieson stain of the wall of the vasospastic basilar artery.

adjacent to the internal elastic lamina. No obvious change was found in the adventitia, although its width seemed to be increased slightly. A mural thrombus was not found in the vasospastic artery by serial sections (Fig. 5). A complete obstruction of the lumen was never verified in the basilar artery even in cases with the severest degree of vasospasm.



(c) Elastica Van-Gieson stain of the basilar arterial wall. (control)

Vasospasm in the basilar artery was also confirmed by arteriography (Fig. 6). The findings were well correlated with those of the direct observation, although the arterial caliber was not feasible to measure precisely by angiography. However, no apparent caliber change was induced by introduction of contrast material in the arteries.

When a small pial artery over the cerebral convexity was ruptured, the extravasated blood frequently flowed out on the arachnoid membrane without a direct contact with the wall of the arteries. In such cases, no or little vasoconstriction was induced. When the

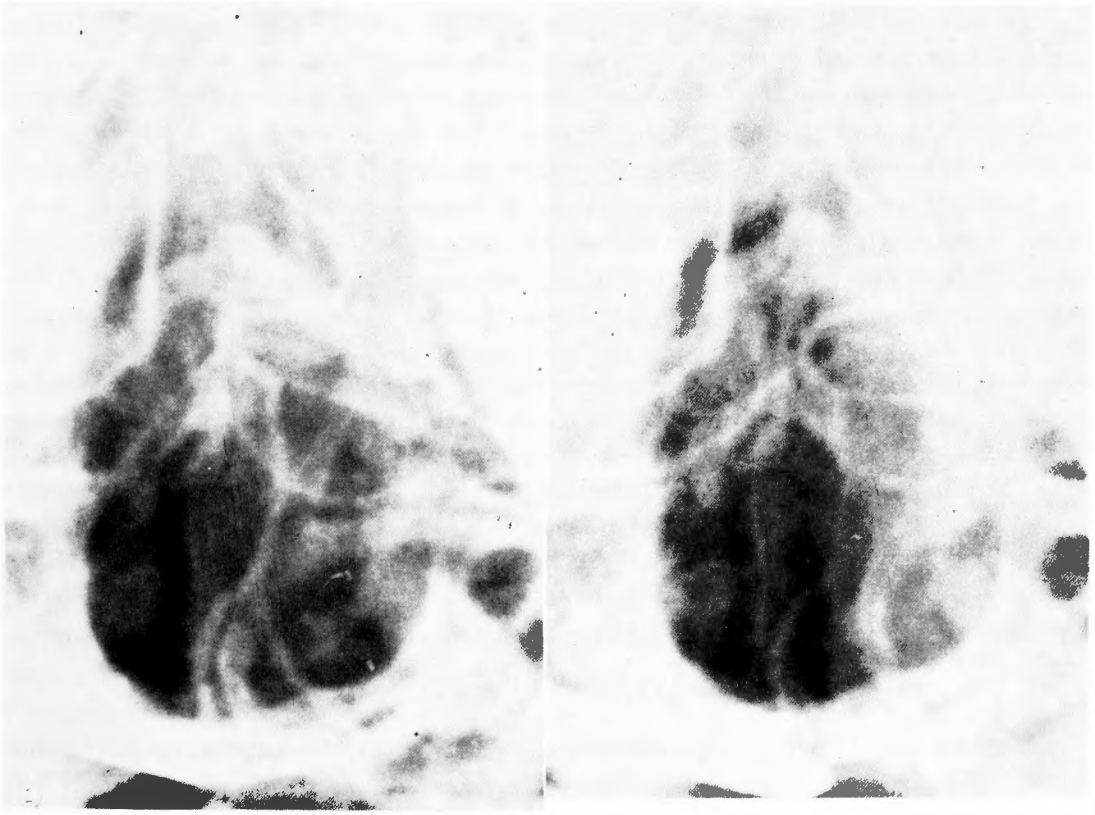


Fig. 6.

(a) Arteriogram of the basilar and vertebral arteries. (control)

(b) Arteriogram of the vasospastic basilar and vertebral arteries.

blood spread along the arteries in the subarachnoid space, a marked constricion occurred usually in the arteries and a marked slugging of the blood flow was visible in the smaller vessels, especially in the venules so that the flow of each red cell could be identified. In the more severe cases of vasospasm the red cells were separated from the plasma and aggregated with an almost complete stasis of the blood flow. Some venules and arterioles were collapsed with a total pallor of the vessel. Although the circulation of the most of the vessels recurred within 30 minutes in most cases, some of the vessels remained collapsed throughout the observation.

Comment ; In the previous experiment Part 1, it was shown that vasospasm caused by a mechanical stimulation continues for a short time. In the present experiment it was shown that spasm did not occur at the time of bleeding when the vessels were under the effects of various mechanical factors, such as a traction of arachnoid bands, or an intraluminal pressure change and etc. These findings indicate that mechanical factors play little role for the production of vasospasm even in its initial stage.

On the other hand, the present experiment demonstrated that vasospasm always occurred by the direct contact of the extravasated blood with the arterial wall. This may indicate that vasoconstrictors in the blood play an important role for the production of vasospasm.

Taveras and WOOD[48] have stated that contrast material may cause a sufficient mechanical or chemical irritation to provoke a contraction of the artery inducing an arterial narrowing temporarily at the injection of a contrast substance. This possibility is denied by the present experiment in which vasospasm was not produced by contrast material in the arteries irrespective of the control arteries or those bathed with the fresh blood.

Symon[47] has thought that a severe arterial narrowing may be produced by some factors other than a smooth muscle contraction, amongst which the most important factor seems to be either an intravascular aggregation or mural thrombus formation. This assumption may be compatible with the finding that the white streaks of the arterial wall were observed to be greatly widened in the vasospastic artery (Fig. 3b). However, as above-mentioned, neither thrombus nor intravascular aggregation was proved histologically in the vasospastic basilar artery and an increase of white streaks was due to an increase in thickness of the arterial wall. Furthermore, no thrombi were found in the basilar artery in cases in which vasospasm lasted for more than 12 hours in the following experiment Part 4, although the degree and the extent of vasoconstriction were proportional to the extent of the morphological changes such as increased convolution of the intenal elastic lamina. Intravascular aggregation developed only in the small venules or arterioloes. Such intravascular aggregation is not therefore concerned with the production of vasospasm.

PART 3. VASOCONSTRICTIVE PROPERTIES IN THE FRESH BLOOD

Method ; The animals were bled via a short polyethylene cathetor in the femoral artery into the tubes containing heparin. The tubes were immediately cooled in ice. The heparinized blood was separated into various fractions by means of centrifugation in refregerator. All glasswares used in the preparation were silliconized.

1) Heparinized whole blood ; The blood drawn from the femoral artery is contained

with heparin in the ratio of 100 unit to 10 cc of blood.

2) Intact platelets in plasma ; The heparinized blood was centrifuged in the speed of 800 r.p.m. for 15 minutes. The platelet-rich-plasma was decanted from the sedimented red cells. The avarage platelet count was 340,000.

3) Lysed platelets in saline ; The platelet-rich-plasma was further centrifuged by 800 r.p.m. for 5 minutes so that no sedimentation of red cells was seen. The platelet-rich-plasma free from the red cells was then centrifuged by 2,500 r.p.m. for 15 minutes and the sedimented platelets were collected. Thus the collected platelets sticked together and could not be evenly resuspended in saline. The platelets were mixed with saline of approximately twenty times volume of the platelets. The membrane of the platelets was destroyed by freezing and thawing. : The tubes were placed in the alcohol cooled to minus 78°C and then replaced in the warm water at 37°C. These procedures were repeated three times.

4) Plasma : The heparinized blood was centrifuged 2,500 r.p.m. for thirty minutes and its supernatant was used.

5) Red cells in plasma without being washed : The heparinized blood was centrifuged 2,500 r.p.m. for 30 minutes and the packed red cells were pipetted from the bottom.

6) Packed red cells : The intact red cells were pipetted from the bottom of the centrifuged heparinized blood and washed by saline by means of centrifugation three times.

7) Lysed red cells : The red cells prepared as in (6), were lysed by freezing and thawing as described in (3).

8) Serum : The fresh arterial blood withdrawn from the femoral artery was stored at room temperature for one hour and the supernatant was pipetted.

The prepared materials were stored in a refregerator and then warmed to the body temperature at the time of experiments.

Each testing material was dripped through a 18 gauge needle on the exposed basilar artery. In five minutes after the application, the testing substance was carefully removed by a siphonage action of the cotton pladget and the photographs were taken. The field was washed repeatedly by warm saline solution until the artery returned to the normal size and was stabilized. Usually four or five different fractions were tested in the same basilar artery.

In order to study persitency of vasospasm induced by the vasoconstrictor substances in the fresh blood, the vasospasm induced by incision of a small branch artery was followed sequentially for twelve hours and the calibser change of the basilar artery was plotted in the graph.

result : The caliber of the exposed arteries changed spontaneously about 10 % in range. Since the reactivity of the arteries to a fraction slightly reduced after occurrence of a severe vasospasm, the order of application of different fractions was changed to avoid the difference of the reactivity of the arteries in each cat.

The results in the application of various fraction of the blood to the artery were summarized in the Table 2. A vasoconstrictor function was almost negligible in the plasma fraction. No vasoconstrictor were found both in the intact red cells fraction and the fraction of the intact red cells without being washed. The vasoconstrictor activity

Table 2 The caliber change of the basilar artery following application of various fractions of the fresh blood

	Average	−60% −50% −40% −30% −20% −10% 0 +10% +20%
Heparinized whole blood	−22%	
Plasma	− 2 %	
Lysed platelets in saline	−21%	
Platelet rich plasma	−18%	
Serum	−20%	
Packed red cells	− 2 %	
Red cells suspended in plasmal	− 3 %	
Lysed red cells	−23%	

was demonstrated in the lysed platelets in saline solution, the intact platelets in plasma, the serum and the lysed red cells (Fig. 7). However, these fractions were milder and more variable in the grade of their vasoconstrictor activities than the fresh blood or subarachnoid bleeding. Vasospasm produced by the fresh blood or subarachnoid bleeding was far more extensive, although it was found to relax to the normal caliber within three hours in most cases, but in a half of the cases vasospasm recurred in the blood-stained part of the vessels within three hours after the relaxation, and the cats died (Table 3).

comment : In 1961 RAYNOR[44] reported that a severe vasoconstriction was produced by a topical application of the serotonin solution to the pial vessels of the cerebral convexity in monkey. From this finding he proposed that spasm might be caused by serotonin which is released from the platelets in the course of the blood coagulation. On the other hand, ECHLIN[15] reported that there might be present much more potent vaso-

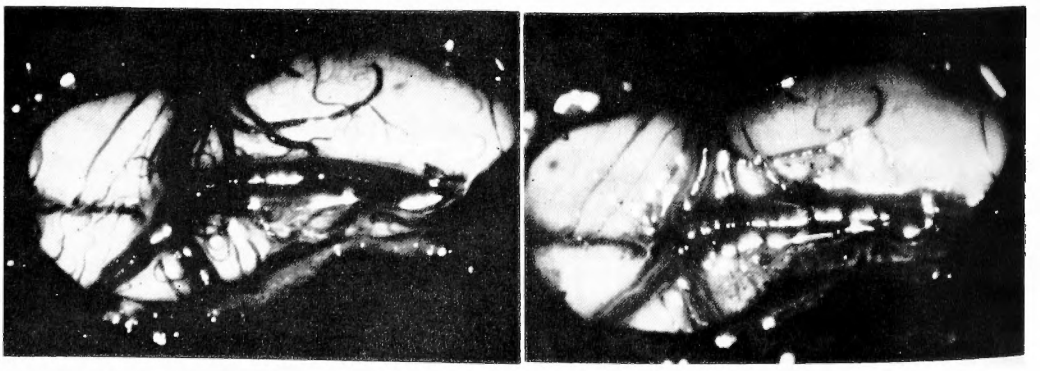
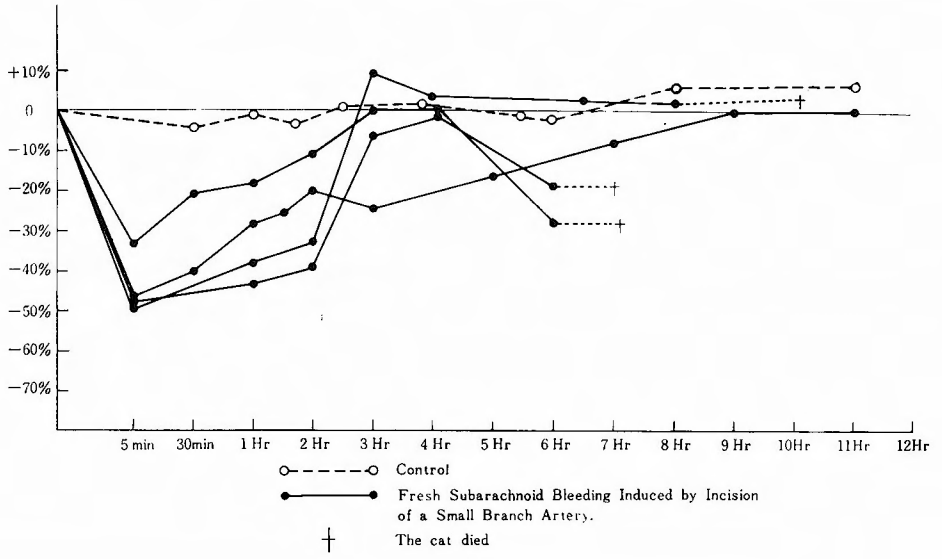


Fig. 7.

(a) The basilar artery (control).

(b) Vasospasm caused by topical application of the fresh lysed red cells.

Table 3 Sequential change in the caliber of the basilar artery following fresh subarachnoid bleeding



constrictor than serotonin in the fresh blood.

In the present experiment, intact red cells did not show any vasoconstrictor activity. In the fear that some vasoconstrictor substance coating the red cells might be washed away by saline solution, red cells not wahed by saline solution was tested for their vasoconstrictor activity, but this possibility was found to be unlikely. Red cells released vasoconstrictor substances when they were lysed. However, the vasoconstrictor in the red cells does not work in the fresh blood, since hemolysis does not occur immediately after subarachnoid hemorrhage.

Vasoconstrictor activity was present in the lysed platelets in saline and the intact platelets in plasma fractions, whereas it was almost negligible in the plasma. Serum contains the vasoconstrictors which are expelled from the platelets in the process of coagulation. Separation of the leucocytes has not been performed in the present experiment because of a technical problem. However, the activity of vasoconstriction in the leucocytes seem to be negligible, for there was no significant difference in the grade of vasospasm between the heparinized whole blood and the lysed platelets fraction which was almost free from the leucocytes.

From the above-mentioned analysis, it is concluded that the vasoconstrictor in the fresh blood is derived from the platelets.

However, the vasospasm with fresh blood was more extensive than that with the platelets fractions. This discrepancy in the grade of vasospasm is considered to be resulted from the partial destruction of the vasoconstrictor in the platelets during the procedure of separation, since the vasoconstrictors were found only in the fractions which contained the platelets or the substances expelled from the platelets. In fact, incubation deprives the platelets fraction or the serum of their vasoconstrictor activity, as will be shown in the

following experiment part 1. This implys that the vasoconstrictors in the platelets are easily degenerated and loose their activity. Furthermore, the vasospasm with fresh subarachnoid bleeding, in which the vasoconstrictors in the platelets were considred to be the cause, relaxed within three hours in the present sequential observation of the spasm. This means that the platelets vasoconstrictors were broken down and ceased to work. KAPP[28] proposed that spasm due to the fresh subarachnoid bleeding would last as long as the clinical vasospasm, but his observation for 90 minutes seems to be too short to cover the relaxation of the spasm.

Since persistent "clinical vasospasm" usually lasts for two to three weeks, some other more stable vasoconstrictor must be searched for.

PART 4. VASOCONSTRICTIVE PROPERTIES IN THE STORED BLOOD

Method : The fractionation of the blood was performed in the same way as in the experiment Part 3. The tubes were kept at 37°C for more than 24 hours to 7 days at maximum. In order to avoid evaporation of the water and contamination of bacteria, the tubes were sealed by Parafilm. The plasma, the intact platelts in the plasma, the intact red cells, the lysed red cells and the serum were tested.

The lysed red cells and the intact red cells were incubated to see the change of the pigment in the red cells for 6 hrs, 24 hrs, 2 days, 4 days, 6 days, and 8 days respecitively and all of them were stored in a dry-ice box. Later, the absorption curves of all these samples were recorded with a spectrophotometer.

Vasospasm induced by the stored lysed red cells was followed sequentially for more than 12 hours. The basilar artery was studied histologically after death of the animals.

Results : The serum and the intact platelets in the plasma lost their vasoconstrictor activities after incubation. The plasma and the platelets in the plasma showed a mild vasodilator activity after incubation. The vasoconstriction due to the lysed red cells was not reduced, but rather increased after incubation. The intact red cells showed a moderate to severe vasoconstrictor activity with hemolysis of a significant degree after a prolonged

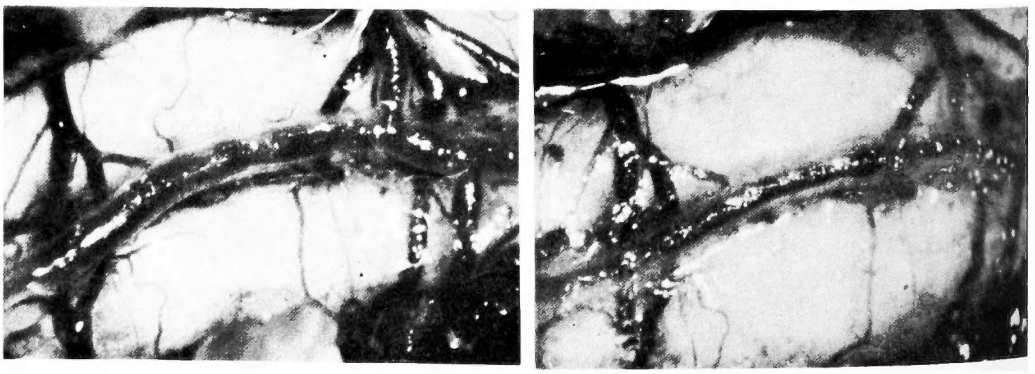


Fig. 8.

(a) The basilar artery (control).

(b) Vasospasm was produced by topical application of the red cells incubated for 7 days.

Table 4 The caliber change of the basilar artery following application of the incubated various fractions of the blood

	Average	−60% −50% −40% −30% −20% −10% 0 +10% +20%
Plasma	+6%	
Platelet rich plasma	+14%	
Serum	−1%	
Packed red cells	−29%	
Lysed red cells	−30%	

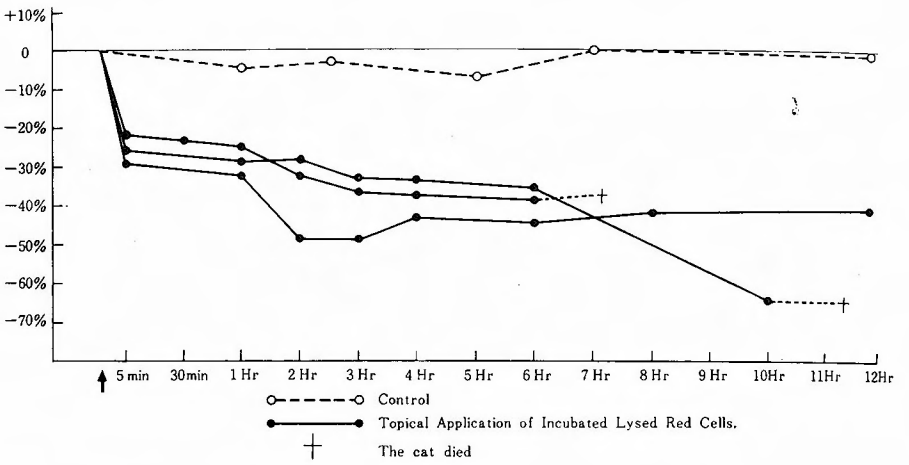
Table 5 Percentage increase or decrease in the caliber of the basilar artery following topical application of the incubated various fractions in relation to duration of incubation.

Plasma									
Case number	221	213	214	208	204	223	216		
Duration of incubation (day)	1	2	3	5	5	6	7		
Percentage increase or decrease in caliber	+13	−8	+20	+4	+14	0	+11		
Platelet rich plasma									
Case number	221	213	214	222	223	224	216		
Duration of incubation (day)	1	2	3	3	6	6	7		
Percentage increase or decrease in caliber	+4	+4	+10	0	+26	+40	+10		
Serum									
Case number	221	213	214	222	223	223	215	216	224
Duration of incubation (day)	1	2	3	4	6	6	7	7	7
Percentage increase or decrease in caliber	0	+6	0	0	−8	0	0	0	−4
Lysed red cells									
Case number	221	233	234	222	237	223	224		
Duration of incubation (day)	1	1	1	3	3	6	7		
Percentage increase or decrease in caliber	−44	−33	−25	−49	−30	−35	+5		
Packed red cells									
Case number	204	217	221	213	213	214	219	222	
Duration of incubation (day)	1	1	1	2	2	3	3	3	
Percentage increase or decrease in caliber	−32	−37	−11	−43	−46	−30	−29	−68	
Case number	204	223	227	205	215	216	224		
Duration of incubation (day)	5	6	6	7	7	7	7		
Percentage increase or decrease in caliber	−14	−24	−15	−22	−29	−40	−14		

incubation (Table 4 and 5). The lysed or intact red cells were separated into two layers after incubation, the reddish supernatant, and the intact red cells and/or ghost red cells. A complete mixture of the two layers were usually applied to the basilar artery. In some cases, only the reddish supernatant was applied, but the degree of th vasoconstriction was almost the same with that of the mixtured.

Vasospasm induced by the incubated lysed red cells increased in severity in the course of 12 hours' observation. No release of vasospasm was observed to the death of the animals (Table 6). No mural thrombus was comfirmed in the vasospastic arteries by

Table 6 Sequential change in the caliber of the basilar artery following topical application of the incubated lysed red cells



histological examinations after the death of the animals.

A typical curve of oxyhemoglobin was depicted by means of spectrophotometer in the fresh intact and lysed red cells. However, in the sample tested 48 hours after incubation, 630 substance was demonstrated in a small amount and gradually increased in its volume during incubation, although oxyhemoglobin was still the dominant pigment even in the sample of 8 days' incubation.

Comment : The instability of vasoconstrictors in the platelets suggested in the part 3 was comfirmed by the findings that the serum and the intact platelets in the plasma lost the activity of vasoconstriction after incubation. Therefore, the platelet-induced vasospasm can not be accepted as the cause of a longstanding vasospasm and some breakdown products of the blood clot must be postulated.

BRAWLEY[8] suggested that some substances with vasoconstrictor might be released in the course of fibrinolysis of the blood clot. However, most vasoactive substances which are released in fibrinolysis are vasodilators and no potent vasoconstrictors have yet been found[47]. The present experiment indicates that the lysed red cells have a stable vasoconstrictor by which a prolonged vasospasm can be induced. Such breakdown products of the red cells have never been taken up as the cause of vasospasm.

Red cells are not hemolysed at the time of bleeding into the subarachnoid space[4)33)]. Accroding to Adams et al.[1], most of the extravasated red cells remain in the subarachnoid space and are gradually destroyed and phagocyted, although some of them are absorbed directly into the systemic circulation in their intact form. DUPONT et al.[13] reported that all the red cells remained in the intracranium and were hemolysed whereas the plasma was rapidly absorbed into the systemic circulation. Many other authors also report that hemolysis occurs twelve or fourteen hours after rupture of an aneurysm, increases gradually its intensity to reach its maximum at about 3 to 5 days after the episode[4)22)33)34)]. ALLCOCK[2] and WILKINS[51] also reported that vasospasm was absent in the arteriograms performed soon after an aneurysmal rupture or operation, but arteriograms done a few

days later revealed an apparent vasospasm.

Above-mentioned facts indicate that vasospasm must be considered at two stages ; 1) short initial stage which is produced by a vasoconstrictor of the platelet origin, and 2) the longsttanding late stage which is caused by a vasoconstrictor released from the red cells. The late stage of vasospasm begins with the development of hemolysis of the extravasated red cells and lasts for a long time until the breakdown products of red cells are cleared by phagocytosis from the vessel wall. This late stage of vasospasm is probably correspond to the "clinical vasospasm", which is found in arteriograms performed more than several hours after the ictus in human cases.

On the other hand, ZUCKER[53] mentioned that an activity of vasoconstrictor in the lysed red cells amount to only 1/80 comparing with that in platelets. However, the lysed red cells, when applied in a large quantity, can induce a severe prolonged vasospasm. When the cerebrospinal fluid is grossly bloody, only 100,000 erythrocytes are usually contained[49)50], whereas about 10,000,000 erythrocytes are present where blood clot surrounds the artery. This fact is compatible with the findings that the most severe degree of vasospasm is usually found around the ruptured aneurysm.

Regarding the vasoconstrictor in the lysed red cells, hemoglobin and its breakdown products probably play the main role in the production of vasospasm. The supernatant of the lysed red cells which consists mostly of oxyhemoglobin induced a vasoconstriction of the same degree as that induced by "mixed" lysed red cells. The supernatant of the lysed red cells irritates the meninges and causes severe symptoms when introduced into the subarachnoid space, whereas ghost red cells were almost inert[26].

Oxyhemoglobin, methemoglobin and bilirubin are the pigments which are detected in the cerebrospinal fluid in patients with subarachnoid hemorrhage[5)49]. Among these pigments, the breakdown products of oxyhemoglobin seem to have more potent activity of vasoconstriction than oxyhemoglobin per se, since the activity is slightly stronger in the incubated than in the fresh fraction, and vasospasm due to the lysed red cells has a tendency to increase in its intensity in the course of observation.

PART 5. NEUROGENIC FACTORS IN PRODUCTION OF VASOSPASM

Method : Either the bilateral sympathetic superior cervical ganglion, or the bilateral sympathetic stellate ganglion was extirpated. In some cats, the superior cervical ganglion of one side was extirpated. One to two weeks after these procedures, the sympathectominized arteries were studied on their reactions to mechanical stimuli, fresh subarachnoid bleeding, and topical application of the incubated lysed red cells with the method previously described in the experiments, Part 1, 2 and 4. Noradrenalin in the sympathetic nerve endings of the arterial wall was checked histochemically following the FUJIWARA's modification[25] of the original fluorescent method of FALCK[18] and compared with that of the untreated cats.

FALCK's fluorescence method modified by FUJIWARA : Cats were sacrificed by rapid intravenous injection of Nembutal in Massive dosis. Major intracranial arteries were extirpated within 10 minutes after the sacrifice, quenched in isopentane solution cooled at $-80°C$. The cerebral arteries were, then, dehydrated in vacuo at a temperature of

−30°C to −35°C for 5 to 7 days. The fully dehydrated tissue section were exposed to formaldehyde gas at 80°C for one hour in a glass jar containing paraformaldehyde. Then, the tissue sections were infiltrated in vacuo with paraffin at 60°C for 30 minutes. The paraffinized tissue sectioned at the thickness of 8μ was placed on the non-fluorescent slide glass and was mounted with a mixture of Entellan (Merck) and xylene in the same ratio. The fluorescent sections were observed and photographed on the Kodak Tri-x film by use of the fluorescent microscope (Carl-Zeiss). The exciting light was delivered from an Osram high pressure mercury lamp and was filtered through Schott BG 12 and Zeiss 50 as the primary and secondary filters.

Results : Noradrenalin contained in the sympathetic nerve endings was well demonstrated as many green fluorescent dots surrounding the smooth muscle layer, but were never observed within the layer of the smooth muscle. All these fluorescent dots disappeared one week after extirpation of the bilateral superior cervical ganglions (Fig. 10).

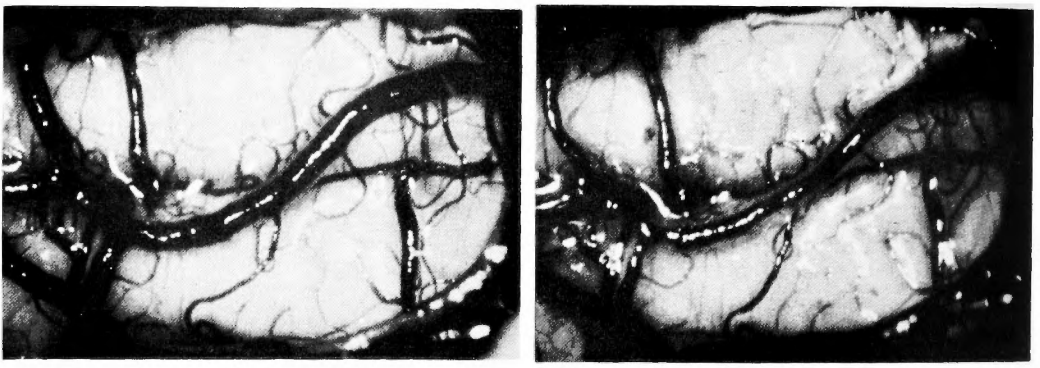


Fig. 9.

(a) The basilar artery (control).

(b) Vasospasm caused by topical application of the lysed red cells incubated for 24 hours in cats whose bilateal saperior cervical sympathetic ganglions were extripated one week before.

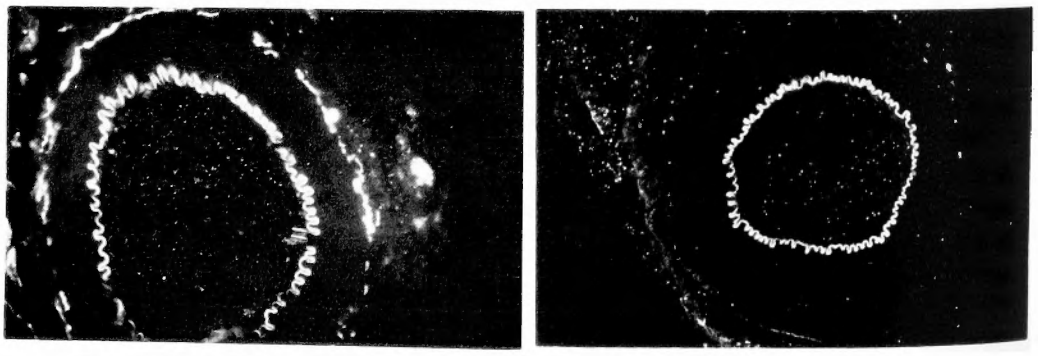


Fig. 10.

(a) The basilar artery (control). Many fluorescent dots of noradrenalin are present in the adventitia, or at the border between the adventitia and the layer of the smooth muscle.

(b) The basilar artery one week after extirpation of the bilateral superior cervical sympathetic ganglion. The fluoresent dots are absent.

In the cases of bilateral stellate-ganglionectomy, noradrenalin fluorescence was found in the intracranial arteries including the basilar and vertebral arteries.

When the superior cervical ganglion was extirpated unilaterally, the complete disappearance of fluorescent noradrenalin was observed in the ipsilateral internal carotid, middle cerebral arteries and their branches, while some remnant dots were recognized in the anterior cerebral artery (Fig. 11). The basilar artery in cats with either bilateral

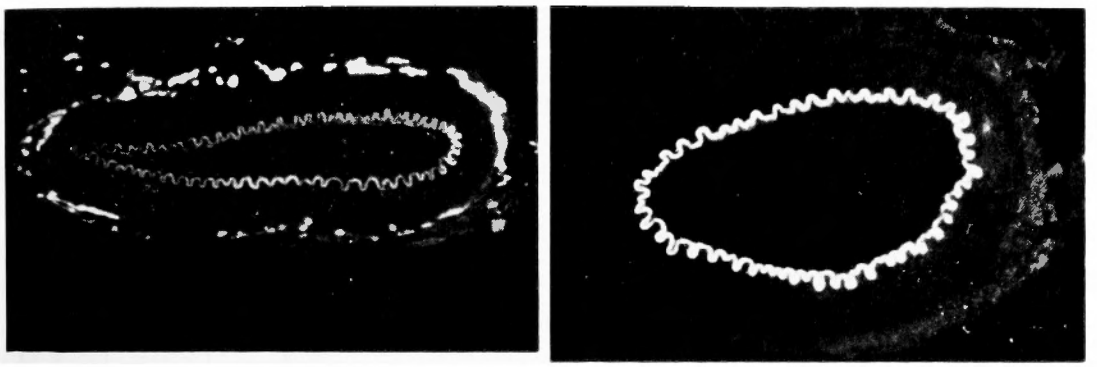


Fig. 11.

(a) The middle cerebral artery with many fluorescent dots of noradrenalin contained in the sympathetic nerve endings (control).

(b) The middle cerebral artery two weeks after extirpation of the ipsilateral superior cervical sympathetic ganglion. The fluorescent dots are absent.

stellate- or bilateral superior cervical ganglionectomy developed vasospasm by means of mechanical stimuli, fresh subarachnoid bleeding or application of the incubated lysed red cells. The degree of vasospasm was almost the same or rather severe than the controls (Table 7).

Table 7 The caliber change of the basilar artery in percentage in cats with sympathetic ganglionectomy.

Extirpation of bilateral stellate sympathetic ganglions			
Case number	225	226	227
Fresh subarachnoid bleeding	−40	−50	
Topical application of incubated lysed red cells	−65	−44	−65
Extirpation of bilateral superior cervical ganglions			
Case number	228	229	
Fresh subarachnoid bleeding		−60	
Topical application of incubated lysed red cells	−48	−60	

The pial arteries in the denervated side also developed vasospasm as well as in the non-denervated side in the cats with extirpation of unilateral superior cervical ganglion.

Comment : The cerebral arteries are richly supplied by perivascular nerves. Although the function of these nerves are little understood, the vasoconstrictor nerves of the cerebral arteries are considered to be the noradrenergic sympathetic nerves which are derived from the sympathetic cervical ganglion[17)41)52)]. Irritation of the perivascular vasoconstrictor nerves has been suggested to cause and extend vasospasm in subarachnoid hemorrhage[19)40)41)46)], and the sympathetic nerve blockade has been tried to treat vasospasm[3)31)35)]. However, since vasospasm usually develops or remains unchanged after the cervical sympathetic nerve block[10)14)30)], there has been controversial opinions on the role of the neurogenic factors for the production of vasospasm. Moreover, none of the experiments have been performed in the denervated animals.

In the present experiment vasospasm developed in the basilar arteries even after the

sympathetic nerve endings in the arteries were deprived of their humoral transmitter, noradrenalin. Therefore, it may be concluded that the neurogenic factor is very doubtful in the production of vasospasm and the vasoconstrictor probably works directly on the smooth muscle of the arterial wall.

Unilateral extirpation of a superior cervical ganglion reulted in disappearance of fluorescence in the internal and the middle cerebral arteries of the same side. This finding implys that the internal carotid and middle cerebral arteries receive the sympathetic nerves only from the unilateral superior cervical ganglion and that there are no interconnecting fibers to the other side of the arteries. The frequent extension of vasospasm to the arteries in the other side[3)43)51)], therfore, may be propagated by extension of the extravasade blood.

GENERAL DISCUSSION

So many factors have been postulated as the cause of vasospasm in subarachnoid bleeding. These include,

(1) Artefact of angiogram……laminar flow[20)].

(2) Organic lesions……mural thrombosis[47)], pressure from the outside of the artery.

(3) Intense contraction of the smooth muscle in the arterial wall.

a) Hyperirritabily of the arteries to contrast material[32)42)].

b) Mechanical factors……Mechanical stimulation at the time of an aneurysmal rupture[10)16)19)23)43)].
Traction of the arachnoid bands due to bleeding[11)27)].
Intraarterial pressure change due to bleeding[43)].

c) Vasoconstrictors in the blood or the breakdown products of the blood.
……Serotonin[44)].
Some unknown vasoconstrictors in the fresh blood[15)28)].
Polypeptides produced by the blood clot lysis[10)51)].
Breakdon products of the blood clot[9)41)43)].

d) Irritation of the periarterial nerves[19)40)41)46)].

Among above-mentioned factors, the possibility of angiographic artefact is denied[33)35)43)]. Mural thrombi were absent in the arteries with vasospasm due to both the fresh subarachnoid bleeding and a application of the incubated lysed red cells. No hyperirritability to a contrast material was found in the cerebral arteries.

The prime importance in the clinical cases in the analysis of vasospasm is the problem of its persistency[7)35)]. In this, mechanical factors which work only for a short time can be excluded. Thus the vasoconstrictors in the blood were investigated and two different vasoconstrictors were found. One is that of the platelet origin and the other is that of the lysed red cells. Vasospasm due to a vasoconstrictr in the platelets lasted longer than that due to mechanical stimuli, but was still too short to explain a longstanding vasospasm in clinical cases. A vasoconstrictor activity in the lysed red cells was very weak when compared with that of platelets. However, such a weak vasoconstrictor, if present in an enoumous amount around the artery, induced a severe vasospasm. Moreover, since the

red cells begin to lyse in the subarachnoid space after a considerable time following subarachnoid hemorrhage, the delayed or longstanding vasospasm may be considered to be caused by the lysed red cells. The role of periarterial nerves in vasospasm is doubtful, for vasospasm developed even in the denervated arteries.

From the above-mentioned systematic analysis of the probable causes of vasospasm, the mechanism of development and maintenance of vasospasm was summarized as shown in the table 8.

Table 8 Mechanism of cerebrovascupar spasm

	Responsible stimulation	Duration	The site stimulated	Remarks
Initial stage of vasospasm	Vasoconstrictor in platelets. (mechanical stimulation ?)	Probably within three hours after the aneurysmal rupture.	Smooth muscle of the arterial wall	Not verified in angiogram unless angiography is performed shortly after the hemorrhage
Late stage of vasospasm	Vasoconstrictor in breakdown products of red cells.	Its appearance is delayed for a few hours, but it lasts for one to three weeks.	Smooth muscle of the arterial wall	"Clinical vasospasm" which is seen in the arterogram in patients with subarachnoid hemorrhage

SUMMARY

A systematic analysis of each proposed factor has been made to elucidate the mechanism of the production of cerebral vasospasm in the basilar arterys and also in the branches of the middle cerebral artery in cats.

1) A severe vasospasm was produced by a mechanical stimulation, a topical application of the fresh blood and the subarachnoid bleeding induced by incision of a small branch artery. The fractionation of the blood was performed, and the activity of vasoconstriction of each fraction was tested. A vasoconstrictor was present in the platelets in the fresh material and in the lysed red cells in the incubated material.

2) The duration of vasospasm was less than thirty minutes in the cases of mechanical stimulations and usually less than three hours in the cases of subarachnoid bleeding. Vasospasm due to the incubated lysed red cells persisted until the death of the animals.

3) The angiographic appearance of the experimental vasospasm correlated well with the findings of the direct observation. No hypersensitivity to contrast material was noted.

4) Histological examinations revealed no mural thrombus in the vasospastic arteries which occurred by the fresh subarachnoid bleeding or a topical apllication of the incubated lysed red cells.

5) Noradrenalin in the sympathetic nerves of the arterial wall disappeared one week after extirpation of the bilateral superior cervical ganglion. Even in such a condition, a severe vasospasm was developed.

6) From the above-mentioned findings, vasospasm is considered to consist of two stages, the brief initial stage due to the vasoconstrictors in the platelets and the longstanding late stage due to an erythrogenic vasoconstrictor. The late stage of vasospasm is considered to correspond with the vasospasm seen in the arteriograms of patients with

subarachnoid hemorrhage. The vasoconstrictors are suggested to work directly on the smooth muscle of the arterial wall.

ACKNOWLEDGEMENT

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和文抄録

脳　血　管　攣　縮

(その発生機序に関する実験的研究)

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学　坂　邦　彦

脳動脈瘤破裂によるクモ膜下出血の予後を左右する因子として，脳動脈瘤破裂部を中心として発生する脳血管攣縮が注目されている．しかしこの脳血管攣縮が発生する Mechanism については，出血による機械的刺激説，Serotonin等の化学物質刺激説，血管周囲神経刺激説，更には血管内血栓等によるとして血管攣縮の存在そのものを疑う説等があり未だに定説を見るに到つていない．

本実験に於ては，猫の脳動脈を用い，脳血管攣縮の原因と考えられている諸因子について系統的検索を行なつた．手術的に露出した脳底動脈及び脳表脳血管を用い，種々の刺激に対する血管の反応を手術用顕微鏡を用いて観察写真撮影を行なつた．

1) 機械的刺激により脳動脈は強い血管攣縮を来たしたがその持続時間は非常に短く，大部分10分以内に寛解した．

2) 脳底動脈上に新鮮血を滴下，又は細小血管枝切断による人工的クモ膜下出血により著明な血管攣縮が発生した．攣縮をおこしている血管を，液体窒素で冷却した Isopentane により急速冷凍を行ない，更に冷凍真空乾燥法により固定後組織検査を行なつたが，血管内に血栓等器質的病変は発見されなかつた．

大腿動脈より大動脈弓にカテーテルを挿入，造影剤を注入して脳血管写を行なつた．脳血管写の所見は直視下の所見と良く一致した．造影剤に対する脳血管の hyperirritability は証明されなかつた．

3) 血液を種々の分画に分離し，各々の血管収縮性を脳底動脈を用いて検討した．新鮮血では，血管収縮性は血清及び血小板浮遊液にあり，血漿には存在しなかつた．正常赤血球は血管攣縮をおこさなかつたが溶血赤血球は血管収縮力を有した．Incubation により血清及び血小板浮遊液はその血管収縮性を失つたが溶血赤血球はかえつてその血管収縮性増大を来たした．Incubation 後，正常赤血球は溶血をおこし，血管収縮性を示した．

4) 新鮮クモ膜下出血及び incubation を行なつた溶血赤血球局所滴下による脳血管攣縮の持続性を12時間以上経時的に観察した．新鮮クモ膜下出血による血管攣縮は3時間以内に寛解したが，溶血赤血球による血管攣縮は観察終了迄持続した．実験終了後脳底動脈の組織学的検索を行なつたが血栓は発見できなかつた．

5) 脳血管攣縮に於ける神経性因子を検討する為に，両側星状交感神経節，又は両側上頸交感神経節を摘除後，脳血管壁交感神経終末枝中の Noradrenalin を Falck の螢光法により検索した．手術後1週間にて両側上頸交感神経節摘除猫では脳血管壁中の Noradrenalin は消失した．この様に，denervate された脳底動脈に於ても新鮮クモ膜下出血及び溶血赤血球滴下により強い血管攣縮が発生した．両側星状交感神経節摘除猫では，血管壁 Nnrdrenalin は消失しなかつた．

6) 以上の所見から脳血管攣縮発生機序は次の如くに考えられる．

脳血管攣縮は血小板性血管収縮物質による初期血管攣縮と，赤血球破壊産物による後期血管攣縮よりなる．血小板性血管収縮物質は不安定で，速かに分解され，初期血管攣縮は約3時間以内に寛解する．後期血管攣縮はクモ膜下出血後，数時間以上遅れて，赤血球の溶血が始まると共に発生するが，赤血球性血管収縮物質は安定であり，長期間持続する．血小板性及び赤血球性血管収縮物質は共に直接脳動脈壁平滑筋に作用すると思われる．

クモ膜下出血患者の脳血管写は出血直後，3時間以内に行なわれる事はまれである．従つて普通脳血管写で証明される脳血管攣縮は赤血球破壊産物による後期血管攣縮であると思われる．